The Transition of Peritoneal Epithelial Cells into Germ Cells in some Amphibia Anura, especially in Rana temporaria.

By

J. Bronté Gatenby,
Exhibitioner of Jesus College, Oxford.

With Plates 21 and 22, and 5 Text-figures.

Introduction.

The theory that the germ cells are derived from the cœlomic epithelial cells, known as the germinal epithelium theory, was advanced by Waldeyer (**1**) in 1870. It has been believed by Kolliker, Balfour, Semon, Semper, and many others. At about the same period (1880) Nussbaum (**2**) advanced a rival theory that germ cells are not derived from the soma, but that they are early differentiated as segmentation products which do not take part in body formation, and do not give up their embryonic character.

Since the discovery of the migration of the primordial germ cells, Waldeyer's theory has lost favour with many embryologists. Beard writes (**3**) : "For myself, in reviewing the actual facts of my observations, I most emphatically endorse the correctness of his (Nussbaum's) brilliant idea. There is no real evidence showing that germ cells are ever formed from any part of the embryo." In another part of his paper Beard makes the following statement: "The transition stages from epithelial cell to germ cell, of which, among others, Semon speaks so confidently without figuring a single one, are in the skate only conspicuous by total absence."

At this juncture it should be pointed out that Beard mentions embryos only up to 42 mm. in length, and he draws none of his conclusions from adult or intermediate ovaries. Not only this, but the demonstration of the migration of germ cells during one short part of any animal's life does not prove that germ cells are never afterwards derived from soma cells.

For the Amphibia Miss King (**4**) has declared in a recent paper on Bufo lentiginosus that transition stages between germ cell and peritoneal cell do not exist. In this she is supported by Allen (**5**). On the other hand, Semon (**6**), Bouin (**7**), Dustin (**8**), Kuschakewitsch (**9**), and Champy (**10**), are all supporters of the germinal epithelium theory. Indeed, it may fairly be stated that most workers on Amphibian germ cells are believers in Waldeyer's theory.

Beard says in his paper: "The change from epithelial cell to germ cell, though asserted times without number, has never really been depicted, and in all probability has never actually been observed. Indeed, it does not exist."

I firmly believe that the peritoneum does produce germ cells, and in this paper I have endeavoured to make good the omission of those of my predecessors who were followers of Waldeyer and Balfour, in publishing drawings of transition stages between peritoneal cell and germ cell, and in bringing evidence of a new character not from the tadpole only, but especially from the adult frog, to show that the epithelium surrounding the ovary is truly germinal.

Whilst admitting that some germ cells possibly migrate from the endoderm of the yolk sac, I feel sure that during life very large additional reinforcements of germ cells arise in the epithelium of the gonad of Amphibia.

It is my pleasant duty to thank Prof. Bourne for allowing me to work in the laboratory during the vacations. Dr.

Jenkinson[1] has helped me greatly by his continual interest in my work. I have also to thank Mr. Goodrich for kind criticism, advice, and encouragement. For the opinions hereafter expressed I am alone responsible.

Technique and Material.

Ovaries of frogs and toads were collected during every month of the year. Bouin, corrosive acetic, strong Flemming and Perenyi were used as fixatives. Sections were cut from 3 μ–9 μ, and were stained on the slide or in bulk with iron hæmatoxylin and orange G, Erlich's hæmatoxylin and eosin, borax carmine or paracarmine, and picro-indo-carmine or picro-nigrosin. Silver nitrate impregnations were also used for whole mounts of epithelium. The eggs, in the ovary of frogs from 10 mm. to 40 mm. in length, were counted under a dissecting microscope—the large ones being removed first, and the smaller ones, after maceration in osmic acid or corrosive sublimate, being shaken and teased apart and counted under a low-power microscope.

Dr. Jenkinson also placed at my disposal a good collection of frog ovaries, and allowed me to examine his fine series of newt, frog, toad, salamander, and axolotl ovaries and testes. Though this paper is mainly concerned with R. temporaria, it should be stated that my remarks and observations for the latter accord in all the main points with Salamandra, Triton (Molge), and Amblystoma, though the series in some cases is not complete enough to allow me to speak so confidently as I feel able to do for Rana. The latter closely resembles Bufo in the history of germ cell production.

[1] Since this was written, news has arrived of Dr. Jenkinson's sad death at the Dardanelles. I shall always be grateful for the privilege I have enjoyed in working under Dr. Jenkinson; his inspiring example will ever remain one of the most valuable benefits I have received during my stay at Oxford, and any small merit which might possibly be attached to this paper is largely traceable to the foundation laid while I was a student in Dr. Jenkinson's class.

THE SPAWNING OF SOME ANURA.

A frog must have some means of providing itself with fresh supplies of eggs when the stock is finished; for instance, a 40 mm. frog or toad, which I believe would be about two years old, has only from 10,000 to 16,000 ova in its ovaries. This figure is derived from a count of the contents of the gonad of September frogs and toads of that length. One must remember that the Anurous Amphibia lay many eggs at a spawning, and were not a means of replenishment at hand the animal, after a small number of spawnings, would be unable to lay further eggs. Frogs and toads have been kept for years and are known to spawn regularly. The following are some figures of the number of eggs laid at one spawning by several species of Anura (Gadow, 'Cambridge Natural History'):

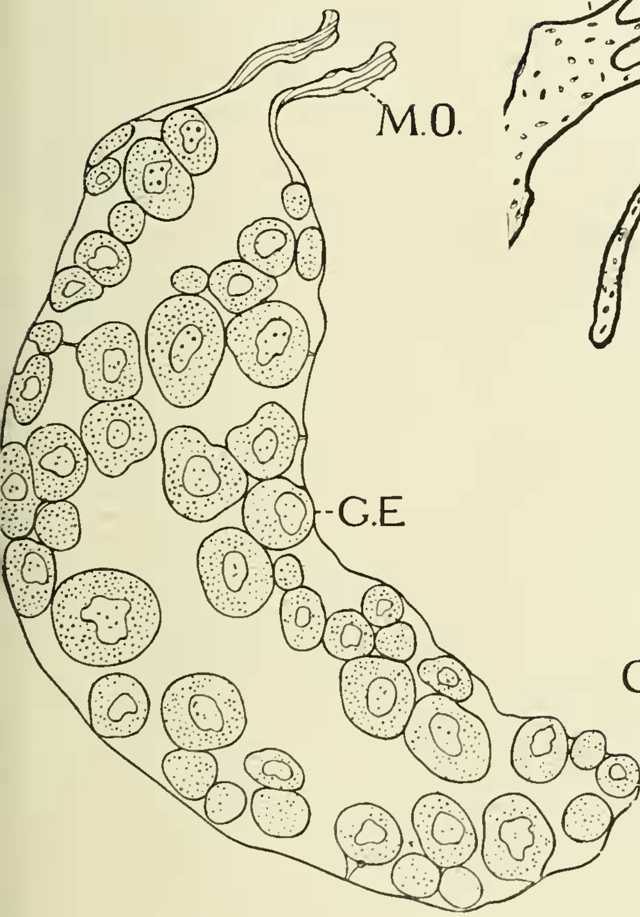
"Hyla arborea 1000; Rana temporaria 3000; Bufo

TEXT-FIG. 1.—Diagrammatic transverse section of ovaries and the surrounding organs in the frog. The dotted line (*P.E.* and *G.E.*) represents the pleuroperitoneal epithelium. That part surrounding and enclosing the ovary (*G.E.*), according to the theory adopted in this paper, is concerned throughout the life of the Amphibian in the production of a constant stream of new germ cells. On the left an egg is drawn diagrammatically to show the mode of attachment to the germinal epithelium by the thecal stalk (*TH. S.*). On the right an ovary is drawn to show the essential arrangement of septa (*S.P.*) and of eggs (*OV.*). *AO.* Aorta. *F.* Follicle, represented by a wavy line. *G.* Gut. *I. V. C.* Inferior vena cava. *K.* Kidney. *M. O.* Mesovarium. *MS.* Mesentery. *OV.* Ovum. *S. J.* Region where septa join the germinal epithelium (*G. E.*). *SP.* Septum. *TH.* Theca. *TH. S.* Thecal stalk or pedicle. *V. C.* Vertebral centrum. [Partly after Bourne (11).]

TEXT-FIG. 4.—Large ovarian germinal area about four or five months old, found in the ovary of an adult frog killed in late May. This thickening hung close up to the inferior vena cava. *M. O.* mesovarium, *R.* refers to Pl. 21, fig. 7, where this region is drawn on a larger scale. This figure is specially drawn for comparison with the next text-figure. Both have been drawn to the same scale with camera lucida.

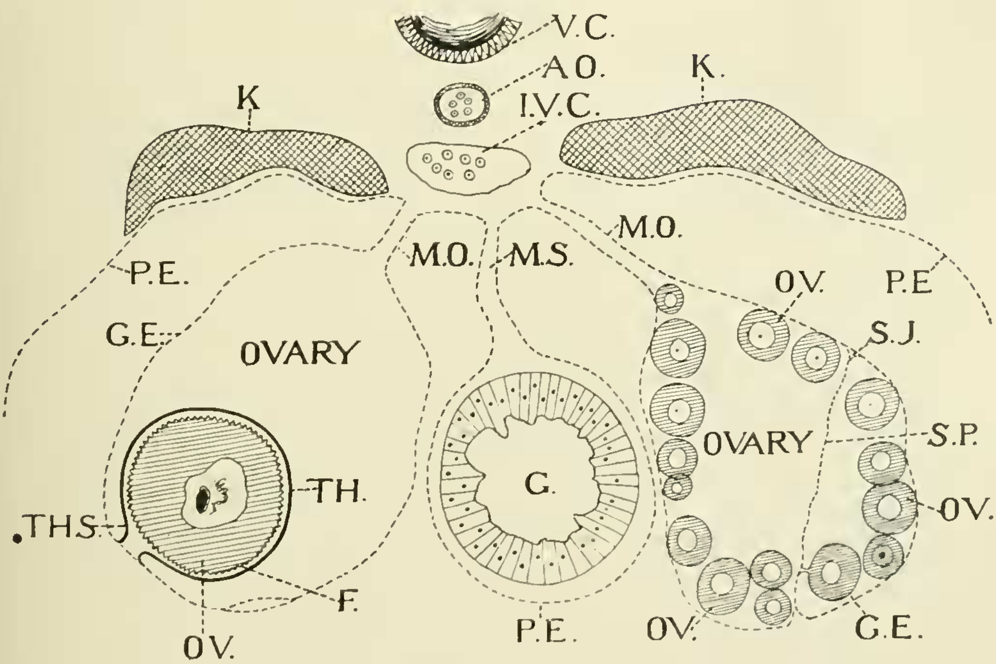
TEXT-FIG. 5.—Ovary of a frog, 40 mm. in length, killed in August. Both Text-fig. 4 and this figure are transverse sections, and serve to draw attention to the fact that in spring and summer the germinal epithelium of the frog ovary contains active germinal islands as large as, and containing more cells than, the ovary of a young frog. These islands are absent in the late winter months.

Text-fig. 5.



Text-fig. 4.

Text-fig. 1.



vulgaris averages 5000; *Rana esculenta* up to 10,000 and more. T. H. Morgan observed a *Bufo lentiginosus* which laid 28,000 eggs within ten hours."

In one of his essays Weismann (**10a**) gives the age to which a toad may grow as forty years. Supposing it began to spawn from four years after birth and kept on till death, it might lay 180,000 eggs during its life. This total is very probably larger than the correct average one, but serves to draw attention to the huge number of eggs some Anura must lay during life. The enormous number of eggs laid at a single spawning by some fish is also a good example of the fertility of some Vertebrata, and illustrates my contention that a germ cell organ must actively work during adult life.

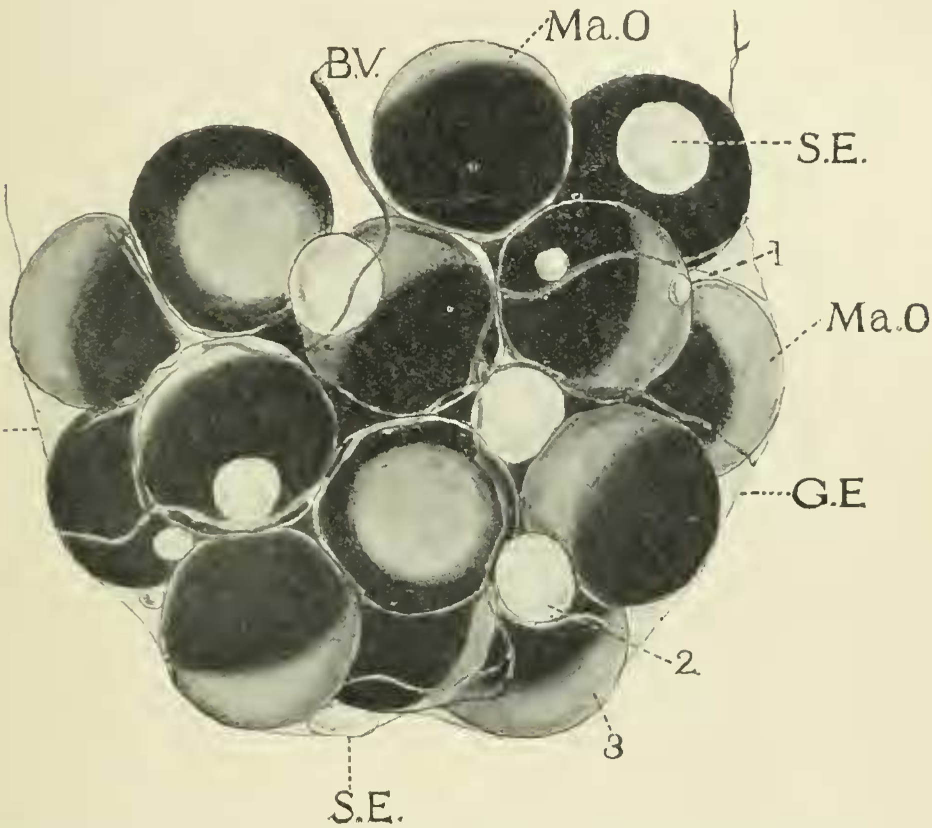
The Germinal Epithelium of R. temporaria and the Changes undergone by it during the Year.

In Text-fig 1 is drawn a diagrammatic section showing the relation of peritoneal epithelium to the viscera. The ova are seen to be suspended in a reflection of the peritoneal membrane, the mesovarium, which "passes right round them," Bourne (**11**). Similarly the gut is suspended by the mesentery. Waldeyer and his followers believe that the part of the peritoneum enclosing the gonad differs from the rest of the pleuro-peritoneal lining in possessing the power of proliferating germ cells. This is denied by Nussbaum's school, which asserts that the peritoneal fold enclosing the ova merely functions as a suspensory sac.

The ova are connected to the germinal epithelium or to the ovarian septa (*S. P.*) by the pedicle of the theca (*TH. S.*). Inside the thecal membrane is the follicle (*F*), which wraps round the ovum itself. The egg thus has two membranes. Blood-vessels connect to the egg from the germinal epithelium by means of the pedicle. Capillaries run between follicle and theca, and by this means every egg is in connection with the blood system.

Examined in winter ovaries, the germinal epithelium is found to consist of a membrane made up of at least two layers of cells, and often as many as three or four. In some places the epithelium is much thickened, the cells being generally about twelve in the layer. There is nothing peculiar in these thickened areas in the epithelium, the

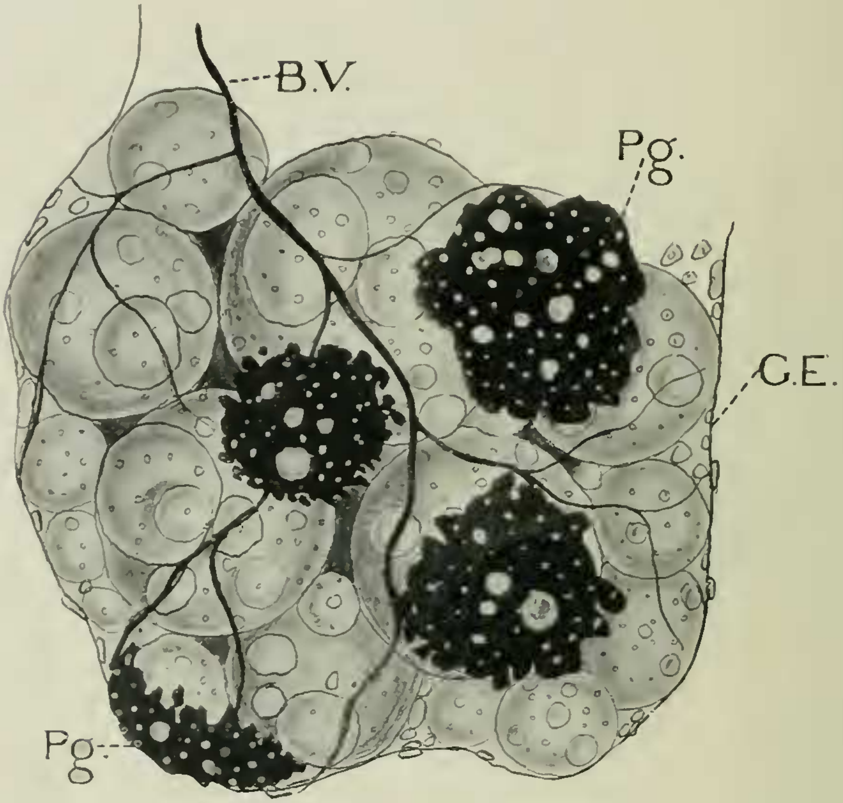
TEXT-FIG. 2.



Part of the ovaryin late December about three months before spawning. The large eggs *Ma. O.* are the ones which will be extruded and spawned. Of the smaller eggs (*S. E.*), which are of different sizes (1, 2), some will degenerate after the large ones have left the ovary, and will form the little black specks drawn in Text-fig. 3, *Pg.* Others will continue to grow during spring and summer after spawning, and will partly be ready for next year's spawning. Those still left in the ovary after spawning are soon reinforced by large numbers of small oocytes derived from the germinal epithelium (*G. E.*), as is shown in Text-fig. 3.

nuclei are not different from those in the ordinary epithelium, and the cell outline, examined in silver nitrate, is found to be more or less wavy, as is characteristic of other peritoneal cells. In Pl. 21, fig. 15, there is drawn one of these thickenings, about four cells in depth. Near *G. E.* the epithelium is of the usual thickness. At the edges where the septa, which form compartments in the ovary, meet the germinal epithelium (Text-fig. 1, *S.J.*), and close up near the insertion of the ovary

Text-fig. 3.



Part of the ovary in May, after spawning. and during the most active period in the history of the ovary. This figure is drawn on a scale about twice as large as that of Text-fig. 2. The black masses (*Pg.*) are degenerating ova of the size drawn in Text-fig. 2 at *S. E.* More especially on the surface of these black masses, but really over the whole surface of the ovary, the germinal epithelium (*G. E.*) is seen to be full of germ cells in every stage of metamorphosis. The new eggs appear more clearly over the pigment masses because of the black background. The edges of the pigment masses are frayed and the process of disintegration is well advanced. Before autumn these masses almost completely disappear. The pigmented hemisphere has not yet appeared in any eggs in this figure.

beneath the aorta, occur large thickenings and areas where numerous cells are collected together. The mesovarian thickenings as well as those near the septa are intended to support and bind the ovary into a compact whole (Text-fig. 4, *M. O.*). Blood-vessels occur here and there over the surface of the epithelium and pass by means of the septa and thecal stalks to the ova.

In the tadpole the peritoneum is formed at an early age from mesodermal cells derived from the retro-peritoneal mass beneath and alongside the aorta or from cells of the mesentery. Most authors are in accord with the conclusion that the peritoneal epithelium is derived from mesoderm, and in view of the evidence brought forward in this paper, the origin of the peritoneum has no small importance.

Text-fig. 2 depicts an ovary in late December.

In the winter months the contents of the gonad consists of very large, almost if not quite, mature ova (*Ma. O.*) and other smaller eggs, the largest of which is generally about one fourth the size of the mature ovum (*S. E.*). The smaller eggs are not pigmented, being a greyish-white in colour.

When spawning time arrives, about March, the large ova are extruded and the ovary shrinks greatly in size, containing only the smaller eggs (*S. E.*) and a few larger eggs not successfully extruded. These latter soon degenerate together with a large proportion of the smaller ova (*S. E.*). In the process of disintegration the inner egg membrane or follicle breaks up, and its cells get mixed up in the lumps of dark yolk (Pl. 21, fig. 6, *F. N.*), and evidently assist in some way or other in removing the waste matter. Very soon the yolk, at first grey, becomes yellow, then brown, and finally turns black. The ovary then has a very characteristic speckled appearance, shown in Text-fig. 3, which is drawn to a larger scale than Text-fig. 2.

The black irregular masses (*Pg.*) are pigment produced from degenerate ova. On them small white masses of various sizes can be seen. These are very young oocytes appearing in the epithelium, which stretches over the black masses. These small white masses appear all over the ovary after spawning, but are seen well only near the pigment masses, since the black background shows them clearly. No pigment has yet made its appearance in the growing eggs in Pl. 21, fig. 3. The pigment appears much later in the year. After spawning the history of the ovary consists roughly in the growth of the remaining oocytes and the formation of others, as explained below.

Towards the end of winter and in early spring the frog emerges from its hibernating place, and if it is not already near a pond or ditch travels towards one. If the ovary at this period, and some time before spawning, be examined the eggs will be found to be similar in size to those described above for late December. It is in the germinal epithelium that changes have taken place already, or are about to occur, and this period in the history of the germinal epithelium is of great importance.

If the epithelium be examined under a low power and attention be concentrated on any thickened areas, many germinal islands, such as is drawn in Pl. 21, fig. 11, will be discovered. The appearance of these islands and their staining reactions are so characteristic that one cannot overlook them. They are swollen areas in the epithelium, containing several cells with nuclei larger and staining less chromatically than the ordinary peritoneal cell nucleus. In the latter the chromatin is gathered in irregular lumps not apparently connected by a continuous reticulum, and a nucleolus can often be discovered towards the middle of the nucleus (Pl. 22, fig. 19).

The chromatin stains darkly with nuclear stains. This description would apply equally well to the follicle and thecal nuclei, which are evidently of quite the same nature and origin as those in the peritoneum. In Pl. 21, fig. 11, there will be seen three nuclei marked *T. S.* The right hand one is bilobed. Each of these nuclei stains less chromatically than the other peritoneal nuclei in the thickening, and, what is more important, each has a distinct granular protoplasmic zone around its periphery. This zone tends to give the nuclei such a curious appearance that their instant detection is not difficult. Viewed from surface preparations of the whole peritoneum, the nucleus and granular area appears as in Pl. 21, fig. 9 (*G. A.*). Occasionally, nuclei may be found in the peritoneum which stain as lightly as those in Pl. 21, fig. 11, but without the zone. These latter are not destined to become germ cells unless a granular area can be discovered around them. I have looked for this characteristic nucleus and granular zone in several other Amphibians. It is very clear in Salamandra and the Axolotl. That this peripheral granulation is derived from the nucleus appears probable, both because it stains with chromatin dyes and because it first appears closely applied around the nuclear outline.

The fact that the zone at first stains clearly in chromatin dyes also suggests that it might be the expelled chromatic material of the nucleus, which latter, it may be remarked, is now becoming more and more faintly staining.

These cells containing nuclei wrapped around by a granular zone become characteristic of the ovary about spawning time and soon cause numerous thickened germ pockets to appear. The further history of these future germ nuclei will be resumed at a later stage.

The Histological Condition of Germinal Thickenings and the First Traces of their Appearance.

Germ islands vary greatly in size. Some are large tracts plastered on the side of the ovary and, after staining, quite visible to the naked eye.

Very large thickenings, such as that depicted in Text-fig. 4, never appear in other parts of the peritoneum except near the mesovarium (Text-fig. 1, *M. O.*) or near the septal joinings (Text-fig. 1, *S. J.*). In all probability the reason for this is that no other part of the peritoneal membrane is strong enough to support such a large area as that of Text-fig. 4. When one remembers that the germ island in Text-fig. 4, could be easily seen by the naked eye and was almost as big as the whole ovary of a 40 mm. frog (Text-fig. 5), this reason will be better understood. The thickening in Text-fig. 4 would be about five months old, assuming that it arose in January or thereabouts. The largest oocytes in it are approximately the same size as the largest oocytes in a five-months-old tadpole ovary.

More commonly ovarian thickenings, instead of containing many thousands of differentiating germ cells, often only contain from twenty to a hundred. One most curious fact must be mentioned with regard to many of the smaller thickenings—it is, that some of them bear such a remarkably complete resemblance to the tadpole ovary that one would have difficulty in distinguishing them were examples of each set side by side under two microscopes. In the later embryonic ovary yolk discs may be present, these never appearing in germ islands.

We speak of the genital ridge of a tadpole. The germinal epithelium of the adult frog develops every year hundreds of genital ridges, some so large that the whole transverse section of an eight-day tadpole would hardly cover them. The migration of primordial germ cells is a curious and interesting fact, but when compared with the annual production of germ cells from the peritoneal epithelium it falls into insignificance.

In the early spring there will be found on the peritoneal epithelium little undifferentiated thickenings such as drawn in Pl. 21, fig. 15. The nuclei composing these areas are quite similar in all details to the ordinary peritoneal nuclei, and the cell wall is wavy in outline, as is usual with peritoneal epithelium. From their subsequent history it seems certain that generally the inner cells of these thickenings metamorphose into germ cells. The outer ones form ovarian membranes—follicle and theca—after the germ cells have metamorphosed into oocytes. In the first instance, these undifferentiated islands arise through the rapid division of pre-existing peritoneal nuclei. Whether this division is mitotic or amitotic is a moot point, but my observations lead me to think that the divisions are amitotic. I do not intend to bring forward my evidence for this in the present paper.

However, the nuclei of the cells in these thickenings do certainly arise from peritoneal nuclei, have the same chromatic arrangement, stain in exactly the same manner, and in short allow of no avoidance of the truth that they are somatic and not germ cells. The germ cell nucleus has a different chromatic arrangement from that of the peritoneal, thecal, or follicle nucleus, and it is quite easy to discriminate between these nuclei.

I wish to make an important point of this, because it is anticipated that attempts will be made to show that the peritoneal islands contain undifferentiated stored-up germ cells which have been dormant till the need for them arose. It is, therefore, very fortunate that almost all ovaries taken in winter have no properly differentiated germ islands, and not only this, but in spring all the intermediate stages between peritoneal cell and germ cell are beautifully apparent in suitable material.

The following changes are undergone in the transformation of the somatic cell into the germ cell. These are the much-discussed stages whose presence has been so confidently denied by Beard and others, and which were believed in by Balfour and Semon.

The peritoneal nucleus has the structure depicted in Pl. 22, figs. 17 and 18, and Pl. 21, fig. 16.

The filaments and lumps of chromatin are coarser in some nuclei than in others. A karyosome is nearly always present, and there are often two. At no stage in the history of the first primordial germ cell is the arrangement of chromatin, such as that of the peritoneal nucleus to be discovered. The shape of the nucleus, its staining power and chromatic arrangement are highly characteristic of the germinal epithelial cell. Not only this, but the cell outline is extremely characteristic examined in silver nitrate preparations. The very earliest sign of a change in the nucleus is detected in a slight loss of shape. The outline becomes less even and somewhat polymorphic. Such nuclei are drawn in Pl. 21, figs. 16B, 16C, and Pl. 22, fig. 20.

The nucleus at this stage grows larger, and little by little loses its staining power (Pl. 22, fig. 21; Pl. 21, fig. 16D). The chromatic lumps and the karyosome do not take the stain so sharply as in other unaffected nuclei, and consequent upon this loss of staining power comes the appearance of the granular zone around the periphery of the nucleus (Pl. 21, fig. 16C and fig. 11).

Step by step as the nucleus loses its staining power the peritoneal cell cytoplasm, before clear and staining exclusively with plasma dyes, becomes more and more granular. The loss of staining power seems not only traceable to the chromatic network, but also to the nuclear sap, or fluid contents of the nucleus (see Pl. 22, figs. 17, 18, 19, 20, etc.).

If the ovary be preserved in corrosive acetic and stained in borax carmine and picro-indigo carmine the peritoneal cytoplasm stains pale greenish blue, the granular zone pink, and the nucleus a little darker, while red blood corpuscles stain dark red in the nucleus and orange yellow in the cytoplasm. In iron hæmatoxylin the granular zone is picked out, even after prolonged differentiation (Pl. 21, fig. 12). With Erhlich's hæmatoxylin the zone appears a light reddish purple (Pl. 21, fig. 16C). At this stage the future germ cells begin to lose their flattened shape, and tend to form cysts as drawn in Pl. 21, fig. 14 or fig. 12. Cyst formation is just beginning in fig. 11; certain peritoneal cells quite near are already becoming turned in the correct direction (*P. C.*).

During the loss in staining power of the future germ nucleus the chromatin lumps seem to become more finely distributed and smaller (Pl. 22, fig. 20). At the same time a faint reticulum can be made out. Whether this was present before the loss of staining power began and invisible because of the coarseness and denseness of the chromatin, or whether it appears secondarily, I cannot decide for certain. In some nuclei at this period a reticulum is quite clear, while in others it seems less easily demonstrable.

In Pl. 21, figs. 16A, B, C, D, are drawn some stages in the transformation of a somatic cell into a germ cell (stained in

Erhlich). The loss of staining power is clearer after using a selective stain like Erhlich's hæmatoxylin and eosin than with iron hæmatoxylin. In Pl. 22, figs. 17, 18, 19, etc., such stages are drawn from iron hæmatoxylin preparations. The nucleoli, after prolonged staining, differentiate quite black. In the polymorphic mulberry shaped germ nucleus (Pl. 22, figs. 22 and 25) many nucleoli may be seen. I believe that these nucleoli may be budded off from the original large nucleoli or nucleolus (Pl. 22, figs. 19 and 21). Each fold of the polymorphic nucleus almost always contains a nucleolus, and very often several. Pl. 22, fig. 24, depicts a different form of nucleus. I think that this is derived from one of the mulberry shaped nuclei, and is really a part of one of the latter after it has divided amitotically into several parts. Later on the reticulum of the nucleus (Pl. 22, fig. 24) becomes coarser, and chromosomes are formed, and a mitotic oogonial division ensues (Pl. 22, fig. 26). In all large germinal thickenings a cavity sooner or later appears and the same process as that undergone in the embryonic ovary takes place—i. e., the ovary, once many oocytes in depth, becomes hollow, and the walls sag down till a depth of only one or two oocytes are found on the walls (Text-fig. 5). During the appearance of the cavity or cavities (for there may be more than one) septa are preserved between the peritoneum and new walls of the cavities, and, these persisting, later on form compartments in the ovary. The lobations and sacculations of the adult ovary are directly traceable to thickenings in the peritoneum, as are also the septal walls of the compartments.

Germ cells do not exclusively appear in the peritoneal epithelium, but they seldom become differentiated inside the ovary on the septa (Text-fig. 1, *SP.*).

In Pl. 21, fig. 6, is drawn a small degenerating oocyte; follicle nuclei (*F. N.*) are mixed up in the pigment masses. The theca is still intact (*TH.*). At *X X* a pocket of cells has appeared. These are future germ cells, and the nuclei have already assumed the irregular shape, and the cytoplasm has become granular. Such nests and cysts as this are not uncommon during the months when many oocytes are degenerating. In fig. 3 of the same plate a part of an April ovary is shown with three old follicle sacs (*F. S.*). In the wall of two of them at *X X* appear several germ cysts. This is quite common in April months. In both this case and the one cited above the germ pocket arises from follicle or thecal cells. In Pl. 21, fig. 6, the new germ cells appear inside the old degenerating oocyte—a remarkable fact.

After spawning many eggs degenerate, as already explained, but those left (Text-fig. 2, *S. E.*) are destined to form the eggs to be laid next year. Though one cannot be certain, I believe that an oocyte takes two years at least, and more probably three, to become mature. It is evident, therefore, that the young oocytes formed in April or May in the adult will not be used for spawning next March, but certainly for a spawning several years ahead. The first ova derived from primordial germ cells would not be spawned till three years after the hatching of the tadpole, since the frogs around Oxford seem to become mature in three years. The tadpole born in March is a metamorphosed frog 25 mm. in length by September. By September of next year it is 40 mm., and by the September of the year after it is almost full grown (70 mm.–80 mm.), and mature enough to spawn. I cannot be absolutely certain for these figures, but they accord with my results for other observations. Very probably the condition of the weather and of the food supply has considerable effect upon the time at which a young frog reaches maturity.

An approximate table of the changes in the ovary of Rana temporaria found around the Oxford district is as follows, but it should be understood that just as the seasons are liable to variation, so are the stages in the cycle liable to be later or earlier :

Winter (January, February, March).—Eggs ready to lay and the germinal epithelium quiescent. Frog hibernating (Text-fig. 2).

Late March or early April spawning takes place.

Spring (April, May, June).—Germinal epithelium becomes by stages very active, many new ova being formed in it. Many eggs degenerate with the appearance of pigment, and the ovary assumes a characteristic speckled appearance (Text-fig. 3).

Summer (July, August, September).—Most of the new germ cells have reached the oocyte stage by August, and a gradual stoppage of activity commences.

Autumn (October, November, December).—The oldest and largest ova are now almost prepared for the spawning of next year, and the activity in the germinal epithelium ceases. Hibernation begins according to the state of the weather (Text-fig. 2).

Bufo spawns later in April.

The Reasons for Believing that the Germ Thickenings Arise from Peritoneal Cells are as follows:

(1) No thickening containing any nuclei except peritoneal ones are to be found in winter. Very few are found in September, fewer in October, and hardly any in November. They are scarcely ever to be discovered in December ovaries.

(2) During early spring the differentiation of the future germ cell island can be clearly followed in all stages.

(3) Thickenings always arise at about the same months (March or April, more rarely February), are commonest in early summer, and begin to disappear towards autumn.

(4) From a count it is clear that the young frog has not enough eggs to supply it through life, and an anatomical and histological examination leaves only the peritoneum from which these new germ cells can arise.

(5) Germ cells may be seen to arise in the follicle sac left by the extruded egg, and must be derived from somatic cells (follicle or thecal) (Pl. 21, fig. 3, *X X*, and fig. 6, *X X*, etc.)

(6) Germ cells may be seen to arise from cells with a typical peritoneal cell outline (Pl. 21, fig. 9).

With regard to the first reason it should be said that frogs hibernating in greenhouses, or during a mild winter, may have small inactive germ pockets. These are so few and the cases are so rare that the above statement is not affected by them. The large majority of winter ovaries have no germ thickenings of any kind, while every spring ovary has many. No exception to the latter statement has been found.

Discussion.

Miss Helen Dean King (**4**) does not mention the germinal thickening in her paper of the "Oogenesis of B. lentiginosus," and I can only infer from this that she has not found them, and, therefore, that she has not needed to explain their presence in terms favourable to the view that at no period in the history of the gonad are germ cells produced by transition from somatic cells. In the tadpole Bouin and Kuschakewitsch both describe the resolution of peritoneal or retro-peritoneal cells into germ cells. Miss King stands alone in her view. I cannot count B. M. Allen (**5**), since his work resembles Beard's in treating of only a short period of the animal's life. Were Allen to search he would probably find other important periods in the history of the sex cells of Rana pipiens. Such evidence for Nussbaum's school is so incomplete as to be useless in a wide review of germ cell production in any one animal.

Beard (**3**) studied the germ cells of Raja, but in his paper on the "Germ Cells" I can find that in no case is any of his evidence derived from adult ovaries. He says regarding his material: "The present researches have been carried out on material of all sorts of phases, from the close of segmentation to embryos of 42 mm., *and they do not extend sufficiently far to permit of the giving of any revised or confirmatory account of the later history,* including the details of the formation of the secondary germ cells. But as Rabl has already said in his own work, so also it is true of mine, that it overlaps the older researches of Semper and Balfour." The italics are mine. From this statement it is very clear that Beard's work on Raja is too incomplete to enable him to advance it as evidence that a study of the germ cells of Raja supports Nussbaum's point of view. Beard shows that according to his own opinion there are no transition stages in embryos up to 42 mm., but he does not, as his discussion, towards the end of his work, would suggest, entirely upset, at one fell swoop, all the edifice built around the germinal epithelium theory. It is extraordinary that Beard should dogmatise on germ cells in general when, by his own showing, his study was not complete enough to "permit of the giving of any revised or confirmatory account of the later history . . . of the germ cells." Even granting that his evidence for this short period in the germ cell history of Raja is correct (which I cannot say), is he justified in condemning Waldeyer until he has examined stages from 42 mm. upwards to maturity, and mature ovaries for every month of the year?

Beard also mentions that a study of the germ cells of the chick supports his views. Compare this with the results since arrived at by Jean Firket (**12**): "Il y a lieu d'admettre une dualité d'origine des gonocytes chez les oiseaux; les gonocytes primaires apparissent très tôt au cours de l'ontogenèse, avant la formation de l'ébunche genital définitive; les gonocytes secondaires se differencient aux depens des cellules de l'epithelium cœlomique qui de ce fait merite le nom d'epithelium germinatif."

Miss King (**4**), in her paper on the "Oogenesis of B u f o l e n t i g i n o s u s," figures in Pl. 1, fig. 4, a germ-cell marked Y, situated outside the gonad. Concerning this she says: "In early stages of development the germ cells are not always confined to the genital ridge," and "such germ cells must eventually come into the germinal area or degenerate, since cells of this character are never found outside of the genital ridge in later stages of development." This germ cell, which is figured outside the genital ridge, is, in my opinion, derived from the retro-peritoneal tissue. One can hardly assume otherwise from the position of the cell.

Though it is impossible to speak for certain in such cases as this, it is most unlikely that these germ cells should have migrated out of the gonad into the position in which Miss King figures them. It seems more natural to suppose that they are developed in situ from retro-peritoneal cells. It is quite likely that some peritoneal cells near the gonad do sporadically become germ cells, and I have found such germ cells outside the gonad in older frogs long after migration had ceased.

Miss King does not explain how the germ cells in her figures came to be in that position. In her fig. 5 of Pl. 1, she depicts other germ cells outside the germinal ridge. Her figures of these stages give no support to the view that the peritoneum does not produce germ cells. In fig. 16 of Pl. 1 she has drawn a young ovary showing the beginning of the formation of the central cavity, and the invasion of retro-peritonal cells described also by Kuschakewitsch (**9**).

The latter claims quite rightly, as I believe, that these cells become germ cells. Miss King, whilst admitting that the invasion of retro-peritoneal cells occurs, prefers to believe that the cells, when arrived in the gonad, form ovarian membrane cells and not germ cells.

During this stage the ovary grows very considerably, not only in size, but in the number of germ cells. Miss King writes: "Although mitotic figures are comparatively rare during the early stages of development they are found very abundantly when the tadpole approaches metamorphosis, and in a single section of the ovary of a toad killed at this time one may find several cells that are preparing to divide (Pl. 1, fig. 17)." In figs. 16 and 17 of Miss King's paper the ovaries depicted are already very large, and no mitotic figures are shown.

Most of the germ cells drawn in fig. 17 in Miss King's paper are not preparing to divide. Though such nuclei as that of fig. 17, *P.*, are present in B. vulgaris and R. temporaria I would not interpret them as "preparing to divide." I have found in the Amphibians above mentioned that the nucleus of the oogonium, when preparing to divide, becomes spherical (not irregular), and the chromatic network resolves itself into a spireme, which then breaks up into chromosomes. Miss King herself was struck by the absence of mitoses during early stages in the growth of the ovary, be it marked, long after the migration of germ cells has stopped; how, then, does she explain the rapid growth of the ovary up to the stage just before metamorphosis, when numerous mitoses begin to appear?

The ovary at this stage is a large organ, and since she denies that amitosis takes place in the germ cells, it is obvious that the rapid growth of the embryonic ovary is due to some cause other than the mitotic division of the primordial germ cells.

Beard mentions the curious absence of the mitoses in the embryonic ovary of Raja. Can it be possible that my remark about Rana applies equally well to Raja?

From my own observations, which, I may say, coincide quite closely at almost all points with those of Kuschakewitsch (**9**) and Bouin (**7**) for the tadpole, I would suggest that the history of germ cell formation in the Anuran is as follows:

In the very young tadpole a few of the yolk sac endoderm cells become transferred to the gonadic region by migrating up a very short path along the mesentery. That this migration does take place in every tadpole I am not sure. It is less easy to be certain of migration in the tadpole than in the Elasmobranch. The number of migratory endoderm cells is very few in almost every case, and the early gonad has hardly ever more than four or five germ cells in transverse section in its broadest part. This remains true for a time, during which migration does no longer take place, since the gut soon becomes quite differentiated. About this time the mesentery and retro-peritoneal cells in the original gonad form the peritoneal epithelium of the ovary, and when the latter constitutes a true continuous epithelium it begins to proliferate germ cells. The invasion of retro-peritoneal cells also sets in, and the gonad grows quickly in size without many mitoses being present to otherwise account for the rapid increase in the number of germ cells. Soon, however, many mitoses appear and many germ cells begin to differentiate. The ovarian cavity forms, and the ovary begins to assume the characteristic shape shown in Text-fig. 5, which is much older than the period to which I am now referring.

By autumn the tadpole born in March has an ovary containing about half the number of ova that, as an adult, it can spawn in one season, and not only this, but proliferation of germ cells from the peritoneum stops about the same time as given in the table for the adult frog (p. 290). We then find that the proliferation of germ cells in the tadpole lasts from about March to October or November (according to the weather), just as it does in the adult. The little metamorphosed frog hibernates, and if in its second year the gonad is examined in spring, a renewal of proliferation of germ cells is detected as in the adult. From birth up to the time of its first spawning, and ever afterwards during life between spring and autumn, the frog has new germ cells formed in its peritoneum. The tadpole ovary, therefore, is formed of:

(1) Germ cells of peritoneal origin.

(2) Germ cells of retro-peritoneal origin.

(3) Germ cells of endoderm (yolk sac) origin. The germ cells derived from the yolk sac may possibly all be laid during the first spawning. The first two origins are mesodermal.

The first germ cells which migrate from the endoderm may be regarded as precocious and relatively unimportant. Through the peritoneum the mesoderm supplies by far the greatest number of germ cells. Endodermal ova, being probably all spawned at the first egg-laying, this spawning consists of a mixed batch of eggs, endodermal and mesodermal in origin. The peritoneal cell always remains in a sufficiently undifferentiated condition to metamorphose, if needed, into a germ cell. The changes undergone in the differentiation of an oogonium into an oocyte are more important and extensive, as far as the nucleus is concerned, than those of the peritoneal cell when becoming a germ cell. We meet with no cataclysmic changes such as the prophases of the heterotypic division, but simply with a loss of staining power, the appearance of a more granular cytoplasm, and the polymorphism of the nucleus.

There is no continuity of germ cells in the Amphibia I have studied—any peritoneal cell apparently can become a germ cell—and why not?

SUMMARY.

(1) Germ-thickenings in the peritoneal epithelium surrounding the ovary do not appear in winter.

(2) In early spring differentiating areas appear in the peritoneal epithelium. Followed out, these thickenings are found to become ovariform germ pockets containing newly formed germ cells in all stages.

(3) The intermediate stages between peritoneal cell and germ cell consist primarily in the elimination of a greater part of the chromatic matter of the nucleus, in the appearance of a granular zone in the cytoplasm which becomes totally granular and stains more heavily than before, and finally in the appearance of several nucleoli subsequent upon a loss of the regular shape of the nucleus.

(4) Intermediate stages have been looked for and found in other Amphibian ovaries.

(5) Thickenings containing many thousands of germ cells and larger than the entire ovary of a lately metamorphosed frog have been found, especially in May.

(6) Reasons are given why the cells composing the germ thickenings in the ovary are not to be considered stored-up germ cells.

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EXPLANATION OF PLATES 21 AND 22.

Illustrating Mr. J. Bronté Gatenby's paper on "The Transition of Peritoneal Epithelial Cells into Germ Cells in some Amphibia Anura, especially in Rana temporaria."

Explanation of Letters used in Figures.

[*a*, *b*, *c*, *d*, *e* refer to stages in transition of peritoneal cell into germ cell as drawn in Pl. 21, fig. 16.]

B. V. Blood-vessel. *C. Y.* Cytoplasm. *F. N.* Follicle nuclei. *F. S.* Follicle sac. *G. A.* Granular area. *G. E.* Germinal epithelium. *L. P.* Leptotene stage. *M. O.* Mesovarium. *N.* Nucleus. *O. M.* Oogonial mitosis. *P. C.* Peritoneal cell. *P. g.* Pigment mass. *T. S.* Transition stage between peritoneal cell and germ cell. *X. X.* Germ pocket *Y. N.* Yolk nucleus. *Y. O.* Young oocyte.

PLATE 21.

[Outline of all figures, except those in Pl. 22, drawn with camera lucida. The arrow points towards the cœlom. Figures reduced by one half.]

Fig. 1.—A lately differentiated germ island in April containing germ cells with mulberry shaped nuclei. Zeiss E. 4. O., oil imm., 2 mm.

Fig. 2.—A germinal thickening a little later in April. There were fifty or more germ cells in this thickening. In the section some leptotene stages are cut across *L. P.* *P. g.* is pigment which is often found near or in thickenings of the peritoneum. Zeiss E. 4. Oil imm., 2 mm.

Fig. 3.—About three weeks after spawning. The follicle sacs here cut across contain in two places little germ pockets *X X*. Zeiss E. 4. O. A. A.

Fig. 5.—A thickening containing three lately metamorphosed oocytes. Zeiss E. 4. Oil imm., 2 mm.

Fig. 6.—The follicle sac of a young degenerate oocyte containing at *X X* a newly formed nest of cells derived from the original follicle or theca. The black mass is due to the degenerating yolk of the disintegrating oocyte. E. 4. Oil imm., 2 mm.

Fig. 7.—The lower part of Text-fig. 1, *R*, drawn to a much larger scale (objective D. D., eyepiece 4); *a*, *b*, *c*, *d*, and *e* stages in transformation of somatic cells into germ cells. E. 4. O.D.D.

Fig. 8.—A part of the mesovarium in which many cells were actively differentiating. E. 4. O.D.D.

Fig. 9.—Surface view of a peritoneal cell. Silver nitrate impregnation. The granular zone, *G. A.*, has begun to appear around the nucleus heralding a differentiation into a germ cell (May). E. 8. Oil imm., 2 mm.

Fig. 10.—A collapsed and partially disguised follicle sac which is beginning to form germ cells in the centre, especially near *X. X.* E. 4. Oil imm., 2 mm.

Fig. 11.—A thickening at a later stage. Granular zone to be seen around the cells marked *T. S.* This thickening is characteristic for April ovaries. E. 4. Oil imm., 2 mm.

Fig. 12.—A thickening in which the germ cells are already beginning to form nests. Late April. E. 4. Oil imm., 2 mm.

Fig. 13.—Lower part of a large thickening showing all stages in the differentiation of a germ cell from a soma cell. The cells opposite the letters *P. M.* are at stage *e* of Fig. 16. E. 4. Oil imm., 2 mm.

Fig. 14.—A nest of germ cells and peritoneal cells in various stages of differentiation. Stage *e* is the furthest, stage *a* the least differentiated.

Fig. 15.—An undifferentiated ovarian island common in the peritoneal or germinal epithelium in early spring. E. 4. Oil imm., 2 mm.

Fig. 16, A, B, C, D, E.—Stages in transition of peritoneal cells into germ cells; stained in Erhlich's hæmotoxylin and eosin in order to show loss of staining power of chromatin. E. 8. Oil imm., 2 mm.

PLATE 22.

(Stained in iron hæmatoxylin.)

Figs. 17, 19, 20, 21, 22, 25.—Stages in resolution of a peritoneal nucleus into germ nucleus. At stage 20 a granular zone had appeared around the nucleus, but it is not shown in the figure.

Fig. 18.—A normal peritoneal nucleus from the same preparation staining almost blackly.

Fig. 23.—A peritoneal nucleus in a stage of losing its staining power.

Fig. 24.—One of the nuclei of Pl. 21, fig. 7. This nucleus has a very fine reticulum, and is in all probability a stage after the formation of the mulberry shaped germ cell (Pl. 22, fig. 25).

Fig. 26.—An oogonial mitosis from one of the germinal thickenings.